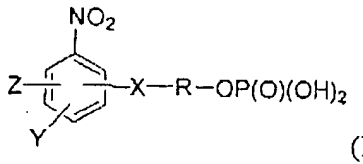
Received 4 July 2005

Amended Claims

1. A phosphate compound of Formula (I)



(I)

wherein:

X represents at any available ring position –CONH-, -SO_2NH-, -O-, -CH_2-, -NHCO- or -$NHSO_2$-;

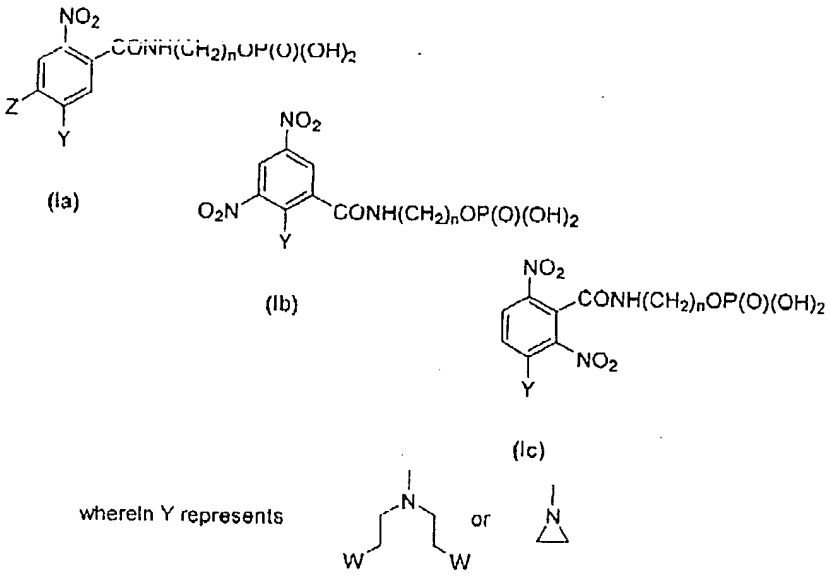
R represents a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom;

Y represents at any available ring position -N-aziridinyl, -$N(CH_2CH_2W)_2$ or –$N(CH_2CHMeW)_2$, where each W is independently selected from halogen or -OSO_2Me.

Z represents at any available ring position -NO_2, -halogen, -CN, -CF_3 or -SO_2Me;

and pharmaceutically acceptable salts and derivatives thereof.

2. A phosphate compound of Formula (I) as claimed in claim 1 which is selected from a compound represented by formulae (Ia), (Ib) or (Ic)



and wherein

n represents 1 to 6

Z represents $-NO_2$, -halogen, -CN, $-CF_3$ or $-SO_2Me$; and

where each W is independently selected from halogen or $-OSO_2Me$

and pharmaceutically acceptable salts and derivatives thereof.

3. The phosphate compound of Formula (I) as claimed in claim 1 or claim 2 which is selected from:

2-[[2-[Bis(2-bromoethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate;

3-[[5-[Bis(2-chloroethyl)amino]-2,4-dinitrobenzoyl]amino]propyl dihydrogen phosphate;

3-[[5-[Bis(2-bromoethyl)amino]-2,4-dinitrobenzoyl]amino]propyl dihydrogen phosphate;

2-[[2-[Bis(2-chloroethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate;

2-[(2-Chloroethyl)-2,4-dinitro-6-[[[2-(phosphonooxy)ethyl]amino]-carbonyl]anilino]ethyl methanesulfonate;

2-({2-[Bis(2-bromopropyl)amino]-3,5-dinitrobenzoyl}amino)ethyl dihydrogen phosphate;

2-[(2-Bromoethyl)-2,4-dinitro-6-[[[2-(phosphonooxy)ethyl]amino]-carbonyl]anilino]ethyl methanesulfonate;

2-[[2-[Bis(2-iodoethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate;

2-[(2-Iodoethyl)-2,4-dinitro-6-({[2-(phosphonooxy)ethyl]amino}carbonyl)-anilino]ethyl methanesulfonate;

2-[(2-Chloroethyl)-2,4-dinitro-3-[[[3-(phosphonooxy)propyl]amino]-carbonyl]anilino]ethyl methanesulfonate;

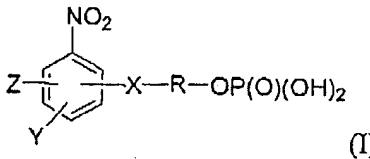
3-({3-[Bis(2-bromoethyl)amino]-2,6-dinitrobenzoyl}amino)propyl dihydrogen phosphate;

2-[(2-Bromoethyl)-2,4-dinitro-3-[[[2-(phosphonooxy)ethyl]amino]-carbonyl]anilino]ethyl methanesulfonate;

2-[(2-Bromoethyl)-2,4-dinitro-3-[[[3-(phosphonooxy)propyl]amino]-carbonyl]anilino]ethyl methanesulfonate; and

2-[(2-Iodoethyl)-2,4-dinitro-3-[[[3-(phosphonooxy)propyl]amino]-carbonyl]anilino]ethyl methanesulfonate.

4. A method of preparing a phosphate represented by the general formula (I);



(I)

wherein:

X represents at any available ring position –CONH-, -SO_2NH-, -O-, -CH_2-, -NHCO- or -$NHSO_2$-;

R represents a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom;

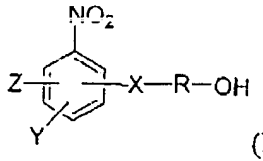
Y represents at any available ring position -N-aziridinyl or -$N(CH_2CH_2W)_2$, where each W is independently selected from halogen or -OSO_2Me;

Z represents at any available ring position -NO_2, -halogen, -CN, -CF_3 or -SO_2Me;

and pharmaceutically acceptable salts and derivatives thereof;

the method including the step of

(i) phosphorylating a compound of formula (II)



(II)

wherein:

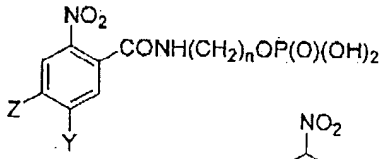
X represents at any available ring position –CONH-, -SO_2NH-, -O-, -CH_2-, -NHCO- or -$NHSO_2$-;

Y represents at any available ring position -N-aziridinyl, -$N(CH_2CH_2W)_2$, or -$N(CH_2CH\,MeW)_2$ where each W is independently selected from halogen or -OSO_2Me;

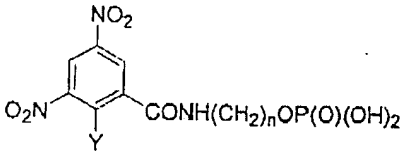
Z represents at any available ring position -NO_2, -halogen, -CN, -CF_3 or -SO_2Me; and

R represents a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom.

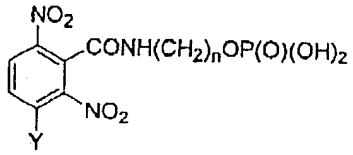
5. A method of preparing a compound of formulae (Ia), (Ib) or (Ic)



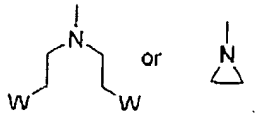
(Ia)



(Ib)



(Ic)

wherein Y may represent 

and wherein

n represents 1 to 6

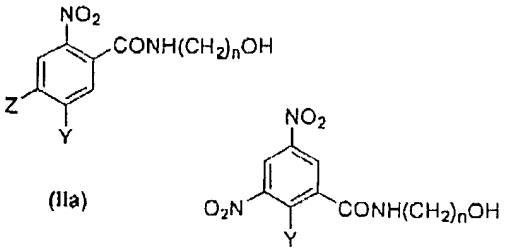
Z represents -NO_2, -halogen, -CN, -CF_3 or -SO_2Me; and

where each W is independently selected from halogen or -OSO_2Me

and pharmaceutically acceptable salts and derivatives thereof

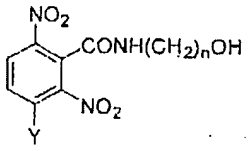
the method including the step of

phosphorylating a compound represented by formulae (IIa), (IIb) or (IIc)

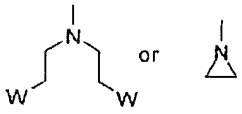


(IIa)

(IIb)



(IIc)

wherein Y represents 

and wherein

n represents 1 to 6

Z represents $-NO_2$, -halogen, -CN, $-CF_3$ or $-SO_2Me$; and

where each W is independently selected from halogen or $-OSO_2Me$

and pharmaceutically acceptable salts and derivatives.

6. A compound of formula (I) when obtained by the method defined in claim 4.

7. A compound of formula (Ia), (Ib) or (Ic) when obtained by the method defined in claim 5.

8. A method of anticancer treatment including the step of administering an amount of a compound of Formula (I) as defined above in any one of claims 1 to 3 to a subject.

9. A method of killing hypoxic cells in a tumour including the step of administering an amount of a compound of Formula (I) as defined above in any one of claims 1 to 3 to a subject with the tumour.

10. The method as claimed in claim 8 or claim 9 including the further step of applying irradiation or one or more chemotherapeutic agents to the subject.

11. The method as claimed in any one of claims 8 to 10 wherein the subject is a human.

12. The method as claimed in any one of claims 8 to 11 wherein the amount administered is between about 20% to 100% of the maximum tolerated dose of the subject.

13. A method of cell ablation utilising at least one nitroreductase enzyme including the step of using a compound of Formula (I) as defined above in any one of claims 1 to 3 in an effective amount to ablate cells which express at least one nitroreductase enzyme.

14. A method of cell ablation utilising at least one nitroreductase enzyme including the step of administering a compound of Formula (I) as defined above in any one of claims 1 to 3 in an effective amount to a subject to ablate cells which express at least one nitroreductase enzyme.

15. The method as claimed in claim 14 wherein the at least one nitroreductase enzyme is encoded for by the nfsB gene of either *E. coli* or by orthologous genes in *Clostridia* species.

16. The method as claimed in claim 14 or claim 15 wherein the cells that express the at least one nitroreductase enzyme are tumour cells in tissue in the subject.

17. The method as claimed in any one of claims 14 to 16 wherein the cell ablation is achieved through GDEPT (gene-directed enzyme-prodrug therapy).

18. The method as claimed in any one of claims 14 to 17 wherein the cell ablation is achieved through ADEPT (antibody-directed enzyme-prodrug therapy).

19. The method as claimed in any one of claims 14 to 18 wherein the cells are mammalian.

20. The method as claimed in any one of claims 14 to 19 wherein the amount administered is between about 20% to 100% of the maximum tolerated dose of the subject.

21. The method as claimed in any one of claims 14 to 20 including the further step of applying irradiation or one or more chemotherapeutic agents to the subject.

22. A pharmaceutical composition including a therapeutically effective amount of a compound of Formula (I) as defined in any one of claims 1 to 3 and a pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser

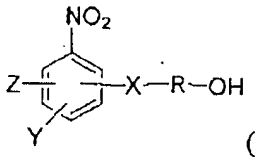
23. The use in the manufacture of a medicament of an effective amount of a compound of Formula (I) as defined in any one of claims 1 to 3 to treat cancer in a subject.

24. The use as claimed in claim 23 wherein the medicament is further adapted for use in cell ablation in conjunction with at least one nitroreductase enzyme including GDEPT (gene-directed enzyme-prodrug therapy) or ADEPT (antibody-directed enzyme therapy).

25. The use as claimed in 24 wherein the at least one nitroreductase enzyme is encoded for by the nfsB gene of either *E. coli* or by orthologous genes in *Clostridia* species.

26. The use as claimed in any one of claims 23 to 25 wherein the medicament is adapted for a mammalian subject.

27. An alcohol compound of Formula (II)



(II)

wherein:

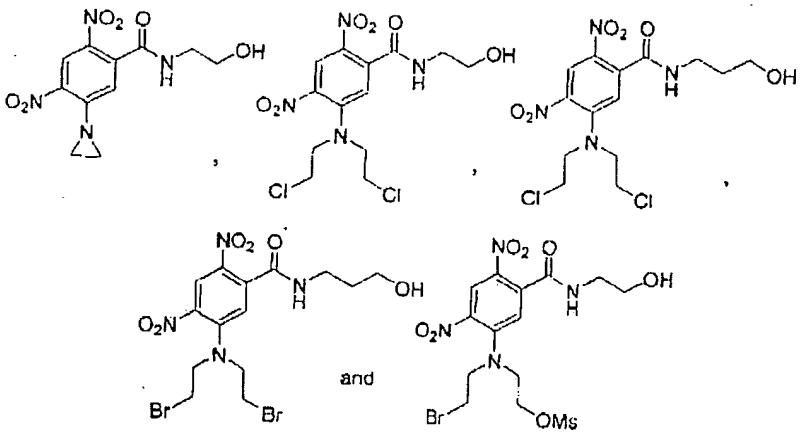
X represents at any available ring position –CONH-, $-SO_2NH-$, -O-, $-CH_2-$, -NHCO- or $-NHSO_2-$;

Y represents at any available ring position -N-aziridinyl, $-N(CH_2CH_2W)_2$, or $-N(CH_2CH\,MeW)_2$ where each W is independently selected from halogen or $-OSO_2Me$;

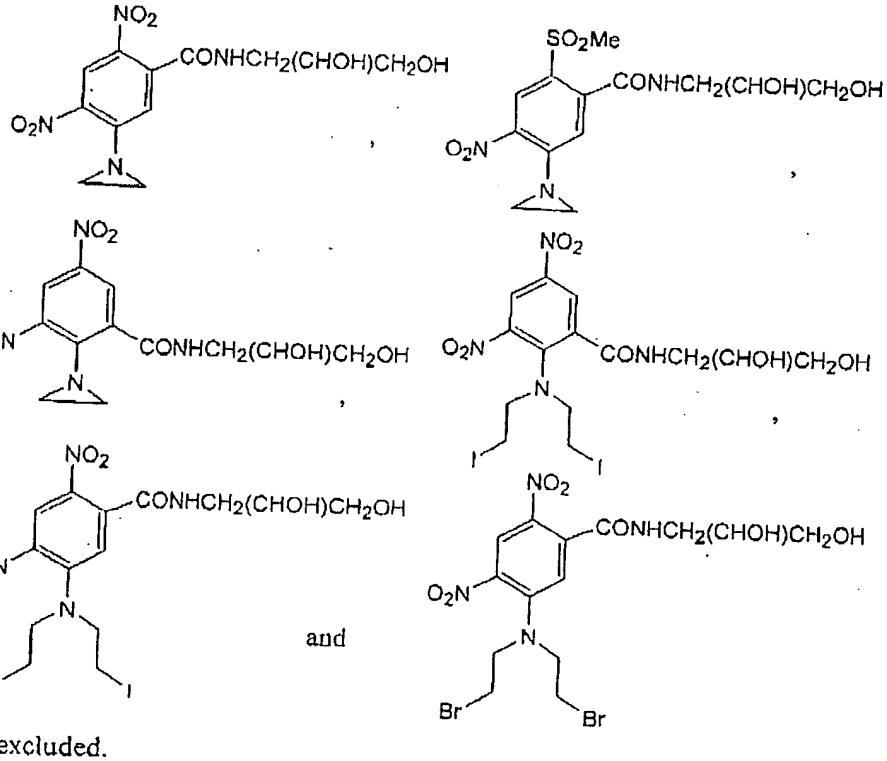
Z represents at any available ring position $-NO_2$, -halogen, -CN, $-CF_3$ or $-SO_2Me$;

R represents a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof, with the proviso that

when Z represents NO_2 and Y represents $N(CH_2CH_2Cl)_2$, X and R together cannot represent -$CONHCH_2(CHOH)CH_2$- and with the further proviso that the following compounds



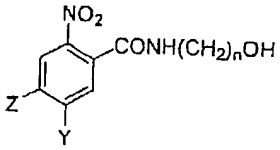
and



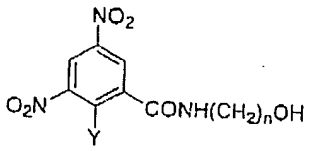
and

are excluded.

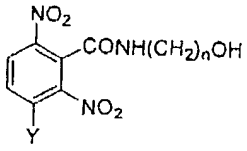
28: The alcohol compound of Formula (II) as claimed in claim 27 selected from a compound represented by formulae (IIa), (IIb) or (IIc)



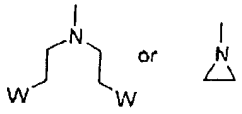
(IIa)



(IIb)



(IIc)

wherein Y may represent 

and wherein

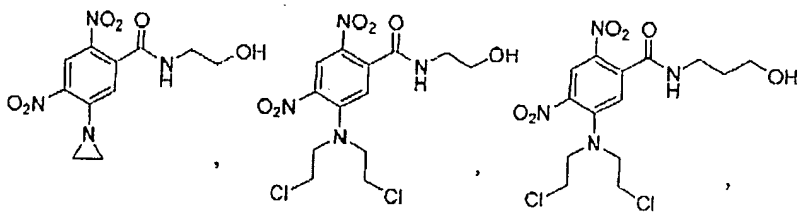
n represents 1 to 6

Z represents $-NO_2$, -halogen, -CN, $-CF_3$ or $-SO_2Me$; and

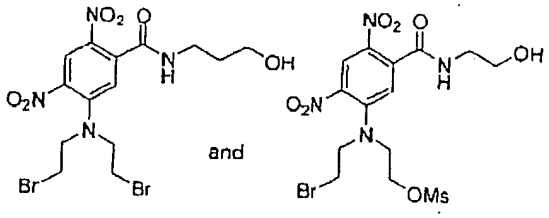
where each W is independently selected from halogen or $-OSO_2Me$

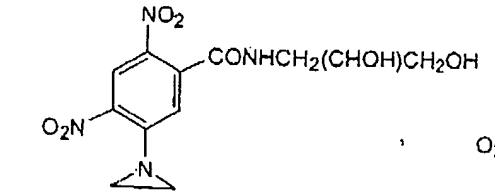
and pharmaceutically acceptable salts and derivatives thereof with the proviso that

when Z represents NO_2 and Y represents $N(CH_2CH_2Cl)_2$, X and R together cannot represent - $CONHCH_2(CHOH)CH_2$- and with the further proviso that the following compounds



and





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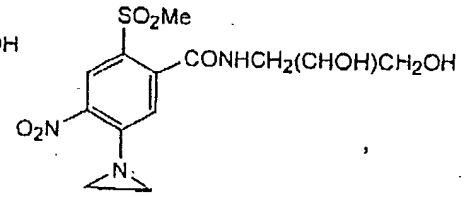

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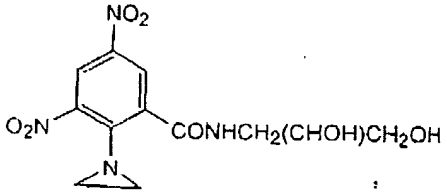

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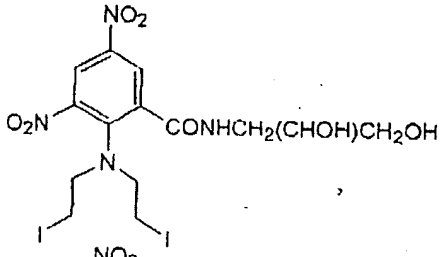

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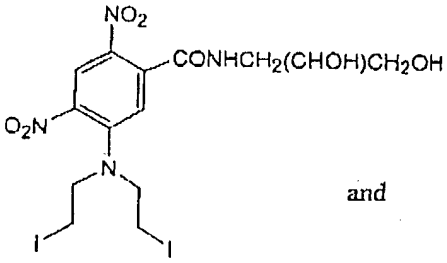

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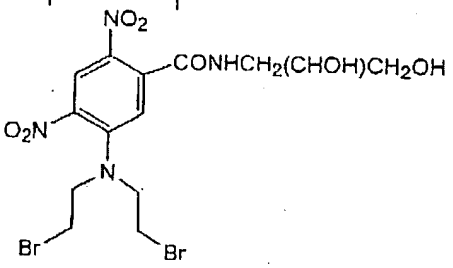

are excluded.

29. The alcohol compound of Formula (II) selected from a compound of Formula (IIb) or (IIc) as defined in claim 28.

30. The alcohol compound of Formula (II) as defined in claim 28 or claim 29 selected from:
N-(2-Hydroxyethyl)-5-[bis(2-bromoethyl)amino]-2,4- dinitrobenzamide;
N-(4-Hydroxybutyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;
N-(5-Hydroxypentyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;
N-(6-Hydroxyhexyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;
5-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-4-(methylsulfonyl)-2-nitrobenzamide;
2[(2-Bromoethyl)-5-[[(3-hydroxypropyl)amino]carbonyl]-2,4-dinitroanilino]ethyl methanesulfonate;
5-[Bis(2-iodoethyl)amino]-N-(2-hydroxyethyl)-2, 4-dinitrobenzamide;
2-[Bis(2-Chloroethyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;
2-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;
2-[Bis(2-chloroethyl)amino]-N-(3-hydroxypropyl)-3,5-dinitrobenzamide;
2-[Bis(2-bromoethyl)amino]-N-(3-hydroxypropyl)-3,5-dinitrobenzamide;

2-[Bis(2-chloroethyl)amino]-N-(4-hydroxybutyl)-3,5-dinitrobenzamide;
2-[Bis(2-bromoethyl)amino]-N-(4-hydroxybutyl)-3,5-dinitrobenzamide;
2-[Bis(2-chloroethyl)amino]-N-(5-hydroxypentyl)-3,5-dinitrobenzamide;
2-[Bis(2-bromoethyl)amino]-N-(5-hydroxypentyl)-3,5-dinitrobenzamide;
2-[Bis(2-chloroethyl)amino]-N-(6-hydroxyhexyl)-3,5-dinitrobenzamide;
2-[Bis(2-bromoethyl)amino]-N-(6-hydroxyhexyl)-3,5-dinitrobenzamide;
2-[Bis(2-bromopropyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;
2-((2-Bromoethyl)-2-{[(2-hydroxypropyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate;
2-((2-Bromoethyl)-2-{[(2-hydroxyethyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate;
2-((2-Chloroethyl)-2-{[(2-hydroxyethyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate;
2-[Bis(2-iodoethyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;
2-((2-Iodoethyl)-2-{[(2-hydroxyethyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate;
3-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-2,6-dinitrobenzamide;
2-((2-Bromoethyl)-3-{[(2-hydroxyethyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate;
3-[Bis(2-bromoethyl)amino]-N-(3-hydroxypropyl)-2,6-dinitrobenzamide;
2-((2-bromoethyl)-3-{[(3-hydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate;
3-[Bis(2-bromoethyl)amino]-N-(4-hydroxybutyl)-2,6-dinitrobenzamide;
2-((2-Bromoethyl)-3-{[(4-hydroxybutyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate;
2-((2-Chloroethyl)-3-{[(3-hydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate; and
2-((2-Iodoethyl)-3-{[(3-hydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate.

31. A method of preparing a compound of formulae (IIa), (IIb) or (IIc)

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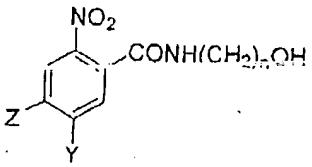
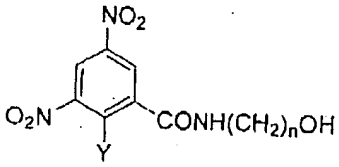
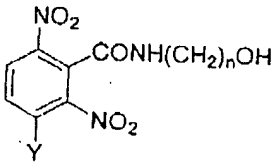


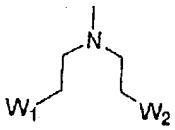
(IIa)



(IIb)



(IIc)

wherein Y may represent 

and wherein

n represents 1 to 6

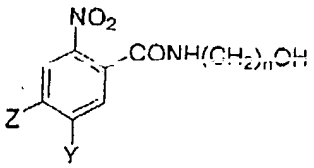
Z represents $-NO_2$, -halogen, -CN, $-CF_3$ or $-SO_2Me$; and

where W_1 is halogen and W_2 is $-OSO_2Me$

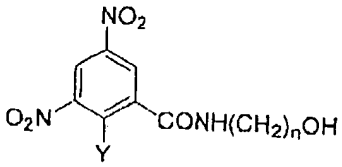
and pharmaceutically acceptable salts and derivatives thereof;

the method including the step of

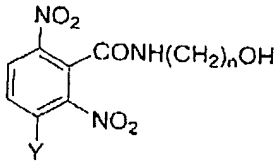
reacting a compound of formulae (IIa'), (IIb') or (IIc') optionally with heating



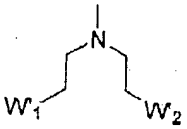
(IIa')



(IIb')



(IIc')

wherein Y may represent 

wherein W'_1 and W'_2 are each halogen;

with an effective amount of silver methanesulfonate (AgOMs) in a solvent to give a compound of formulae (IIa), (IIb) or (IIc) defined above in this claim.

32. The method as claimed in claim 31 wherein the solvent is selected from MeCN or other polar non-protic solvent.

33. A compound of formula (IIa), (IIb) or (IIc) obtained by the method defined in claim 31 or claim 33.

34. A method of anticancer treatment including the step of administering an amount of a compound of Formula (II) as defined in claim 27 to a subject.

35. A method of killing hypoxic cells in a tumour including the step of administering an amount of a compound of Formula (II) as defined in claim 27 to a subject with the tumour.

36. The method as claimed in claim 34 or claim 35 including the further step of applying irradiation or one or more chemotherapeutic agents to the subject.

37. The method as claimed in any one of claims 34 to 36 wherein the subject is a human.

38. A method of cell ablation utilising at least one nitroreductase enzyme including the step of using a compound of Formula (II) as defined in claim 27 in an effective amount to ablate cells which express at least one nitroreductase enzyme.

39. A method of cell ablation utilising at least one nitroreductase enzyme including the step of administering a compound of Formula (II) as defined in claim 27 in an effective amount to a subject to ablate cells which express at least one nitroreductase enzyme.

40. The method as claimed in claim 39 wherein the at least one nitroreductase enzyme is encoded for by the nfsB gene of either *E. coli* or by orthologous genes in *Clostridia* species.

41. The method as claimed in claim 39 or claim 40 wherein the cells that express the at least one nitroreductase enzyme are tumour cells in tissue in the subject.

42. The method as claimed in any one of claims 39 to 41 wherein the cell ablation is achieved through GDEPT (gene-directed enzyme-prodrug therapy).

43. The method as claimed in any one of claims 39 to 41 wherein the cell ablation is achieved through ADEPT (antibody-directed enzyme-prodrug therapy).

44. The method as claimed in any one of claims 39 to 43 wherein the cells are mammalian.

45. The method as claimed in any one of claims 39 to 44 including the further step of applying irradiation or one or more chemotherapeutic agents to the subject.

46. A pharmaceutical composition including a therapeutically effective amount of a compound of Formula (II) as claimed in claim 27 and a pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser.

47. The use in the manufacture of a medicament of an effective amount of a compound of Formula (II) as claimed in claim 27 as an anticancer agent in a subject.

48. The use as claimed in claim 47 wherein the medicament is further adapted for use in cell ablation in conjunction with at least one nitroreductase enzyme including GDEPT (gene-directed enzyme-prodrug therapy) or ADEPT (antibody-directed enzyme therapy).

49. The use as claimed in claim 48 wherein the at least one nitroreductase enzyme is encoded for by the nfsB gene of either *E. coli* or by orthologous genes in *Clostridia* species.

50. The use as claimed in any one of claims 47 to 49 wherein the medicament is adapted for a mammalian subject.